Supplementary Material S2

Raltegravir Assay Description

An API 5500 mass spectrometer was used in positive mode for all quantitations. A simple protein precipitation method using acetonitrile (AcN) containing raltegravir-international standard (RAL-IS) was employed to extract raltegravir from human plasma. Chromatographic separation was performed on an ACE C_{18} analytical column (50 × 3 mm, 3 µm particle size). The mobile phase consisted of 0.1% formic acid:methanol, run isocratically at a ratio of 42.5:57.5 % (by volume). Detection and quantitation was accomplished by multi reaction monitoring (MRM), and raltegravir and raltegravir-IS were detected using the following transitions for protonated products $[M+H]^+$: *m/z* raltegravir 444.9→108.9 and 444.9→361.0; *m/z* raltegravir-IS 451.0→367.2. The primary channels, *m/z* 444.9→108.9 and 451.0→367.2 were used for quantitation purposes. The assay was linear in the range of 10 to 10,000 ng/mL and had a minimum quantifiable limit (LLOQ) of 10 ng/mL using 10 µL of human plasma.